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Amino Acid Metabolism in Young Pea Seedlings [1, 2]

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Introduction

The most striking feature of the amino acid composition of young pea seedlings, as shown in several contributions from Virtanen's laboratory (21, 22) is the behavior of homoserine. This amino acid, which is a precursor of threonine in yeast (4) is present in very low amounts in the ungerminated seed (3). During the early growth of the seedling homoserine becomes the dominant amino acid but after 10 to 14 days it declines again to a low level.

In the present work a variety of C^{14}-labeled amino acids was supplied to young pea seedlings in order to study the fate of products of protein breakdown. A substantial portion of each of the labeled amino acids was converted to homoserine. To set this information in the general metabolic framework of the seedling, changes in protein, starch and soluble nitrogenous components were measured in the cotyledons and other parts of the seedling. A preliminary report has appeared (9).

Materials and Methods

Pea seeds (*Pisum sativum*, var. Alaska) were obtained from Burpee Seed Company, Clinton, Iowa. Before germination seeds were soaked for 4 hours in deionized water at room temperature, then for 2 minutes in 0.001 M $HgCl_2$ (14). After a 2-minute rinse in deionized water, the seeds were planted in moist vermiculite and kept in the dark at room temperature (22°–26°). Unless otherwise stated, etiolated seedlings were used. For amino acid analysis with a Technicon Auto Analyzer, seeds were treated as above and then placed in a growth chamber at 20°. The sprouting seeds were exposed to a 16-hour light period of 400 ft-c and were watered with a complete nutrient solution as needed.

Plant material injected with radioactive substrates (see below) was supported on a plastic screen fixed above 5 ml of water in a fritted glass filter funnel. CO_2-free air entered through the stem of the funnel and was dispersed by the fritted glass disc into the water. This produced a stream of moist CO_2-free air which constantly flowed over the plant material. Respired CO_2 was carried by the air stream through 5 to 10 ml of $Ba(OH)_2$ in a 50-ml centrifuge tube equipped with a Vigreaux column which was the only outlet from the centrifuge tube. CO_2 samples were collected (as $BaCO_3$) every hour or as desired. The $BaCO_3$ precipitates were centrifuged, resuspended in water and plated on microporous filter discs. The C^{14} content of the $BaCO_3$ was determined on a continuous gas flow GM tube automatic counter.

Radioactive substrates were injected directly into the cotyledons of seedlings with a Hamilton microliter syringe. To facilitate injection the seed coats were removed. Each cotyledon received a single injection of 5 μl of the radioactive solution. During

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the incubation time the fritted glass filter funnel, which served as the incubation chamber, was covered with black paper. The following radioactive materials were obtained from commercial sources: L-glutamic acid-U-C^{14} (specific activity 70.7 mc/mmole), L-aspartic acid-U-C^{14} (59.2 mc/mmole), L-leucine-U-C^{14} (246.0 mc/mmole), and D-glucose-U-C^{14} (3.9 mc/mmole).

Homoserine-U-C^{14} was isolated from pea seedlings which had metabolized L-aspartic acid-U-C^{14} or $C^{14}O_2$. Its specific activity was approximately 0.3 mc/mmole.

At the termination of the experiments the seedlings were usually divided into roots plus shoots (i.e. the embryo proper) and cotyledons. These were killed in boiling 80 % ethanol. The ethanol was decanted and the tissues were ground in a glass homogenizer, then extracted several times with 80 % ethanol. The nitrogenous compounds in this fraction are referred to as soluble-N. The extracts were combined and taken to dryness under reduced pressure at 35°. The residues were extracted with 30 to 50 ml of cold ethyl ether which yielded the lipid fraction. The remaining residue was taken up in 5 to 10 ml of warm water and fractionated into a basic, acidic and neutral fraction on Dowex resins according to the method of Canvin and Beevers (5). The amino acids were found in the basic fraction; the organic acids, mostly acids of the trichloroacetic acid cycle, were found in the acidic fraction, while sugars were retained in the neutral fraction. The basic fraction, containing amino acids, was placed with a few ml of 2 N HCl in a closed screw cap test tube and heated in a boiling water bath for 4 hours. This treatment converted asparagine and glutamine to their corresponding acids. This fraction was then passed through Dowex 1 (acetate form) which made possible the separation of the dicarboxylic amino acids from the neutral and basic amino acids (7).

All fractions were then dried at 35° on a rotary evaporator and redissolved in 25 ml of water. To determine total C^{14} content the organic material contained in several aliquots (1 ml) was converted to CO_2 by persulfate oxidation as described by Katz et al. (8) and modified by Stiller (17). The CO_2 was trapped in 20 % CO_2-free KOH, and $BaCO_3$ was precipitated by adding 10 % $BaCl_2$ in 1 % NH_4Cl solution. Aliquots were also streaked on Whatman No. 3 paper and separated chromatographically in 1 dimension with *N*-butanol-propionic acid-water (26:19:27 by volume) as solvent (2). For the identification of various components, other solvent systems (15) were employed in 1 and 2 dimensional chromatograms. Radioactive compounds were located by autoradiography on Kodak no-screen X-ray film. To determine the percentage distribution of C^{14} in a particular fraction that had been separated by paper chromatography, a strip 1 inch wide was cut out and examined on a Vanguard 880 strip counter. A tracing of the radioactive peaks was obtained. By measuring the area under the peaks, the C^{14} in any one peak was obtained as a percentage of the total radioactivity on the strip. Amino acids, organic acids and sugars which had been separated by paper chromatography were located by spraying the paper with ninhydrin (19), bromcresol green (6) and aniline hydrogen phthalate (15), respectively.

A series of pea seedlings was grown for 25 days to determine the changes in the free amino acids during that time. The basic fractions were isolated, as described above, on Dowex resins. Before separation of the individual acids by column chromatography the basic fractions were taken up in 16 ml of 6 N HCl for hydrolysis. The solution, in a sealed glass test tube, was placed in an autoclave at 121° for 2 hours. The solution was filtered, dried at 35° and taken up in a known volume of water. Aliquots were taken for analysis on a Technicon Auto Analyzer (11). Gradient elution from the exchange column (133 cm) was accomplished with 750 ml of citrate buffer. The initial solution was 0.25 N sodium citrate buffer pH 2.91 and the final solution 2.4 N sodium citrate pH 7.5. The buffer was forced through the heated column (60°) under 60 psi pressure. Elution rate was about 37 ml per hour. An alternative method for the separation of the dicarboxylic amino acid fraction was by gradient elution of this fraction from a 19 x 1 cm Dowex 1 (acetate) column. The eluting solution was acetic acid with a 0 to 4 N gradient (7).

The protein content of the alcohol-insoluble residue of the cotyledon and the root plus shoot was determined by extracting the residue 4 times with 5 ml 0.1 N NaOH. This treatment removed 97 % of the total N as shown by values obtained by Kjeldahl digestion of both the NaOH extract and the residue remaining after NaOH extraction. The protein was then precipitated with a saturated solution of trichloroacetic acid, centrifuged and hydrolyzed in a sealed glass test tube in an autoclave in 15 ml of 8 N HCl for 12 hours at 121°. The hydrolyzate, black with humin, was filtered. The filtrate was evaporated to dryness and the residue taken up in a small amount of water and separated into amino acid and neutral fractions on Dowex resins. Aliquots of each fraction were analyzed as described above. Starch was extracted from the residue by the method of Pucher et al. (12). The purified starch was hydrolyzed in 0.7 N HCl at 100° for 2 and one-half hours. Aliquots of the hydrolyzate were analyzed as above.

Total alcohol insoluble residues and lipids were oxidized with the Van Slyke-Folch reagents in an apparatus described by Stutz and Burris (18). The CO_2 was flushed from the system with nitrogen, collected in $Ba(OH)_2$ as $BaCO_3$, and plated as described above.

For determination of labeling, carbon-1 was removed from glutamic acid with ninhydrin and carbon-5 by the Schmidt reaction (1).

Soluble carbohydrate and purified starch were

determined by the anthrone method (20) and protein nitrogen by conventional micro Kjeldahl digestion with nesslerization of the resulting ammonium sulfate. The method of Yemm and Cocking (23) was used for those amino acid fractions not determined with the auto analyzer.

Results and Discussion

Changes in Seed Constituents during Germination. Figure 1 shows the changes which occurred in the level of protein in the cotyledons and root plus shoot over a 10-day period. Five mg of protein-N (73 % of the total) were lost from the cotyledons and a gain of 1 mg was observed in the seedling proper. Striking increases in the amount of soluble-N occurred in both organs (fig 2). The highest rate of protein hydrolysis occurred between the second and fourth days of germination. This observation determined the choice of 2-day-old seedlings for later experiments in which the fate of added radioactive amino acids was investigated. Table I shows the changes in various measured components during the first 10 days of germination. During this time, roughly one-third of the protein and starch in the original seed had been utilized, and the dry weight of

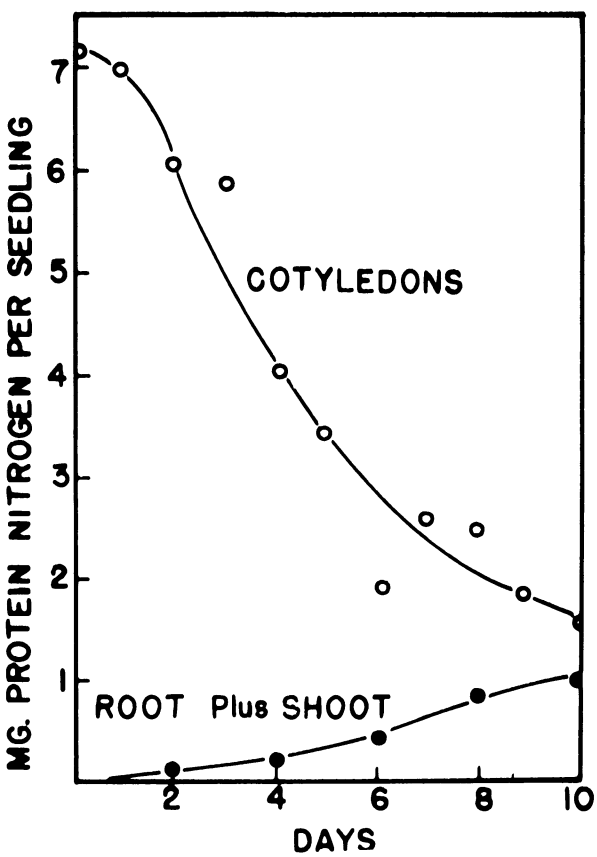

FIG. 1. Changes in protein content of pea seedlings during germination in the dark.

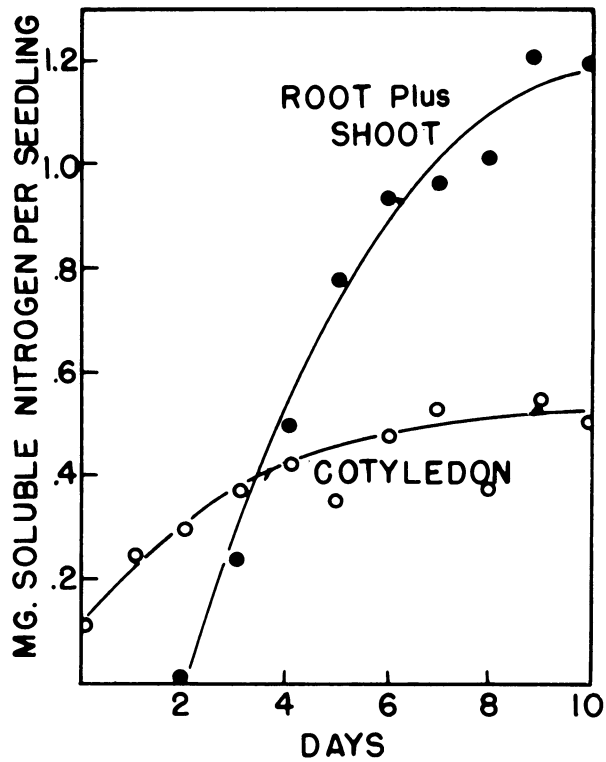

FIG. 2. Changes in total soluble nitrogen compounds in pea seedlings during germination in the dark.

the seedling proper had increased to over 36 mg. Although the soluble-N component increased in both seedling and cotyledons, the total soluble-N of the seedling exceeded that of the cotyledons by the fourth day and the protein soluble-N ratio was considerably higher at all times in the cotyledons.

Further information on the soluble-N compounds was obtained in a series of detailed analyses of green shoots and of cotyledons. These data are presented in tables II and III. The most striking feature is the extremely large amount of homoserine present in the shoots between the third and twelfth days. Homoserine was also the second largest component of the free amino acids in the cotyledons of 2 and one-half day-old seedlings. Although homoserine is present in small amounts in dry ungerminated seed (3) it was not detected in these experiments because of the small amounts of material analyzed. Apart from homoserine, the major amino acids in the green shoot were glutamic and aspartic acids. In general, about 10 amino acids each contributed approximately 1 % or less to the total amino acid content of the green shoots while the cotyledons were relatively rich in these amino acids after germination had begun. Arginine is an extreme example. It could not be detected in the shoot at any stage although at 2 and one-half days it comprised over 11 % of the free amino acid content of the cotyledons. Again proline, glycine and γ-aminobutyric acid are

Table I. *Changes in Composition of Pea Seedlings during 10 Days of Germination in the Dark*

Data in mg per seedling.

	Cotyledon		Root and shoot
	0 Days*	10 Days	10 Days
Residue dry wt**	145	60	25
Protein	44	12	6
Amino nitrogen***	0.1	0.5	1.1
Starch	65	24	...
Soluble sugar	12	4	10

* Data at 0 days includes both the cotyledon and embryonic root and shoot.

** Dry weight after extraction of ethanol soluble fraction.

*** Amino nitrogen in the 80 % ethanol soluble fraction.

notable constituents of the cotyledon after germination but were not found in quantity in the shoots. After this work was completed, a report by Lawrence and Grant (10) appeared. They have provided extensive data on the free amino acids in young seedlings of Alaska pea, variety Unica. The general trends are similar to those reported here, but the absolute values for some of the components are considerably different from ours. Presumably varietal (13) and cultural (16) differences account for this.

The relationships between homoserine, aspartic acid (plus asparagine), glutamic acid (plus glutamine) and how much they contribute to the total amount of the free amino acids in the green shoot as it ages are shown in figure 3. The amount of

Table III. *Free Amino Acids of Pea Seedling Cotyledons*

Data in μmoles per seedling.

Age (Days)	0	2.5	10*	10**
Aspartic†	0.08	0.47	0.49	1.33
Threonine	0.18	0.65	0.31	1.91
Unknown	0.10	...	0.31	1.46
Serine	0.04	1.59	0.81	3.04
Homoserine	...	2.71	0.30	0.59
Glutamic††	0.69	5.76	1.60	1.86
Proline	0.01	0.63	0.14	0.50
Glycine	0.12	0.28	0.28	1.29
Alanine	0.11	0.65	0.26	1.02
Valine	0.03	0.57	0.25	1.06
Methionine	0.01	0.07	0.01	...
Isoleucine	0.01	0.19	0.13	0.58
Leucine	0.02	0.17	0.15	0.67
Tyrosine	0.01	0.08	0.11	0.46
Phenylalanine	...	0.32	0.11	0.38
λ-Aminobutyric	0.01	0.40	0.26	1.07
Lysine	0.01	0.28	0.23	0.86
Histidine	0.01	0.41	0.40	0.49
Arginine	0.01	1.94	0.11	0.05
Ammonia	0.19	3.50	0.73	2.34
Total μmoles†††	1.45	17.17	6.26	18.61

* 10-day-old cotyledons from etiolated seedlings

** 10-day-old cotyledons from green seedlings.

† Aspartic acid plus asparagine.

†† Glutamic acid plus glutamine.

††† Total μmoles does not include ammonia.

Table II. *Changes in the Free Amino Acid Composition of Green Pea Shoots during Germination.*

Data in μmoles per seedling.

Age (Days)	3	5	7	10	12	16	20	25
Aspartic*	0.12	1.89	5.06	11.24	11.28	15.90	6.58	9.74
Threonine	0.06	0.16	0.27	0.35	0.37	0.36	0.35	0.47
Serine	0.09	0.28	0.48	0.75	0.55	0.56	0.70	0.89
Homoserine	3.40	7.75	28.50	24.20	17.88	4.36	1.94	2.30
Glutamic**	0.49	0.90	3.58	3.82	3.81	2.94	3.56	4.78
Glycine	0.05	0.12	0.19	0.36	0.27	0.26	0.31	0.49
Alanine	0.21	0.80	0.99	0.99	0.77	0.49	0.51	0.65
Valine	0.02	0.19	0.36	0.56	0.32	3.36	0.15	0.22
λ-Aminobutyric	T†	0.08	0.16	0.17	0.17	0.12	0.21	0.16
Lysine	0.02	0.07	0.18	0.30	0.40	0.25	0.21	0.21
Histidine	T†	0.08	0.18	0.23	0.55	0.19	0.26	0.37
Other amino acids††	0.06	0.19	0.27	0.55	0.40	0.35	0.44	0.18
Ammonia	0.58	2.40	5.00	10.10	10.10	14.90	7.00	9.10
Total μmoles†††	4.52	12.51	40.22	43.52	36.77	29.14	15.22	20.46

* Aspartic acid plus asparagine.

** Glutamic acid plus glutamine.

† Trace.

†† Proline, cystine, methionine, isoleucine and leucine represent the other amino acids. Each of these amino acids was present as less than 1 % at each age.

††† Total μmoles does not include ammonia.

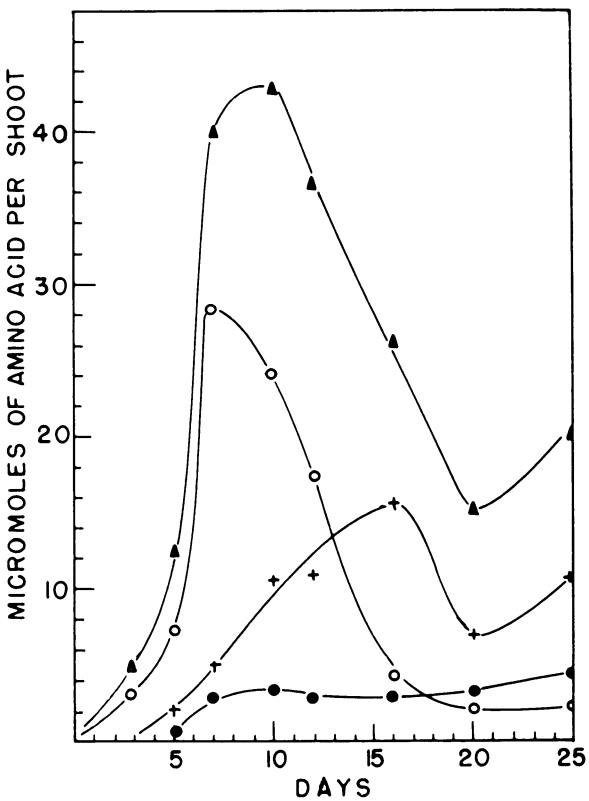

FIG. 3. Changes in some amino compounds in green shoots during germination. ▲ — ▲ Total amino acids. ○ —○ Homoserine. + — + Aspartic acid plus asparagine. ● — ● Glutamic acid plus glutamine.

free amino acid per shoot reached a peak at 9 days and declined quite steeply thereafter. The homoserine curve shows that this is the component primarily responsible for the change. The curve for aspartic acid plus asparagine was the only rising one during which the homoserine level was falling, and separate determinations showed that it was the amide asparagine which was responsible for the increases.

Fate of Amino Acids Supplied to Germinating Seedlings. A) Glutamate: Uniformly labeled glutamate was injected into the cotyledons of etiolated seedlings, and the incorporation of C^{14} into various components of the seedling was measured on samples killed at 3, 6, 12, and 24 hours. The results are presented in table IV.

In 24 hours, less than 4 % of the C^{14} recovered was present in glutamic acid, the supplied substrate. $C^{14}O_2$ was produced from the outset, and one-half of the C^{14} recovered was in this form after 24 hours. By 3 hours C^{14} had appeared in components of the amino and organic acid fractions of the cotyledon. Transport of labeled compounds to the root and shoot was also evident at this time. With time the C^{14} in the organic acids of the cotyledon was depleted so that less than 1 % of the C^{14} recovered

Table IV. *Percent Distribution of C^{14} Recovered after Supplying Glutamic Acid-U-C^{14}*

Glutamic acid-U-C^{14} was supplied to cotyledons of 2-day-old etiolated seedlings which were analyzed 3, 6, 12, and 24 hours after injection. In each of the 4 experiments 5 pea seedlings were used. Each group of 5 seedlings received 0.91 μmole (1.25 μc) of glutamic acid-U-C^{14} in 0.05 ml of solution.

	Percentage of total C^{14} recovered in named component			
	3 hr	6 hr	12 hr	24 hr
Alcohol insoluble residue				
Cotyledon	5	6	5	19
Root and shoot	<1	<1	<1	<1
CO_2	15	22	46	48
Alcohol soluble fractions				
Cotyledon				
Dicarboxylic amino acids and their amides*	43	30	12	4
Neutral plus basic amino acids**	12	19	17	9
Organic acids	15	16	10	<1
Sugars	<1	<1	<1	<1
Ether soluble	<1	<1	<1	<1
Root and shoot				
Dicarboxylic amino acids and their amides*	2	2	2	2
Neutral plus basic amino acids**	3	3	5	13
Organic acids	<1	<1	<1	2
Sugars	<1	<1	<1	2
Ether soluble	<1	<1	<1	<1

* Glutamic and aspartic acids and their amides.

** No less than 80 % of the C^{14} recovered in this fraction was found in homoserine.

was present in that fraction at 24 hours. In the root and shoot tissues the greatest increase of C^{14} with time was realized in the neutral and basic amino acid fractions. The C^{14} in homoserine contributed 90 % of the C^{14} recovered in the neutral and basic amino acid fraction. The increase in labeled homoserine in the root and shoot occurred concomitantly with a decrease in the C^{14} in the amino and organic acid fractions of the cotyledon. At early times (3-6 hours) C^{14} in the neutral and basic amino acid fraction of the cotyledon was largely in γ-aminobutyric acid. At no time did the sugar or lipid fractions account for a significant part of the C^{14} recovered. Some glutamate C^{14} was incorporated into the alcohol insoluble residue of the cotyledon.

B) Aspartic acid: The percentage distribution of C^{14} recovered at intervals after injecting aspartic acid-U-C^{14} is given in table V. Rapid utilization of the aspartate occurred for only 15 % of the substrate, remained unchanged after 3 hours, and the aspartate recovered from the tissue at 24 hours was virtually unlabeled. At 3 hours more

Table V. *Percent Distribution of C^{14} Recovered after Supplying Aspartic Acid-U-C^{14}*

Aspartic acid-U-C^{14} was supplied to cotyledons of 2-day-old etiolated seedlings which were analyzed 3, 6, 12, and 24 hours after injection. In each of the 4 experiments, 5 pea seedlings were used. Each group of 5 seedlings received 0.08 μmole (5.0 μc) of aspartic acid-U-C^{14} in 0.05 ml of solution.

	Percentage of C^{14} recovered in named component			
	3 hr	6 hr	12 hr	24 hr
Alcohol insoluble residue				
Cotyledon	7	7	9	8
Root and shoot	<1	<1	<1	<1
CO_2	16	30	34	36
Alcohol soluble fractions				
Cotyledon				
Aspartic acid	15	7	1	<1
Glutamic acid	9	5	2	2
Asparagine	<1	<1	<1	} 3
Glutamine	4	3	4	}
Homoserine and its lactone	18	17	25	12
Other amino acids	<1	<1	<1	<1
Organic acids	21	18	11	8
Sugars	1	1	1	1
Ether soluble	1	1	2	1
Root and shoot				
Aspartic and glutamic acids	<1	<1	1	1
Asparagine and glutamine	<1	1	<1	<1
Homoserine and its lactone	2	6	5	22
Other amino acids	<1	<1	<1	1
Organic acids	1	1	1	<1
Sugars	<1	<1	<1	<1
Ether soluble	<1	<1	<1	<1
Total cpm recovered (thousands)	723	625	599	759

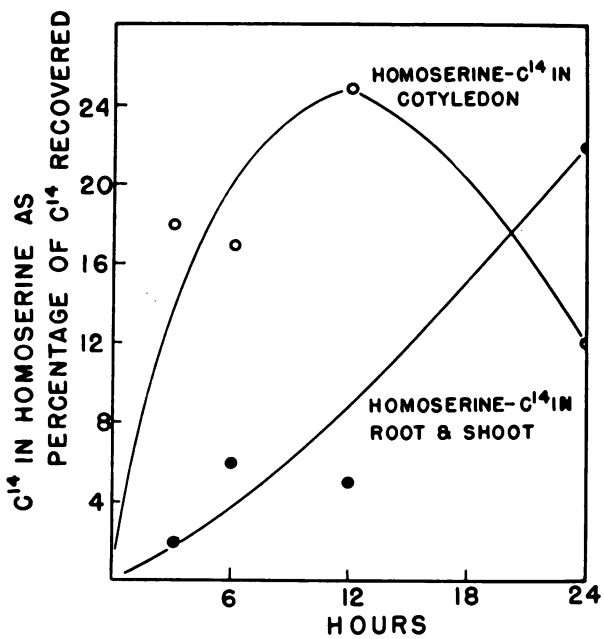

FIG. 4. Changes in the C^{14} content of homoserine (plus lactone) after supplying L-aspartic acid-U-C^{14} (0.08 μmole, 59.2 mc/mmole, in 0.05 ml supplied to 5 seedlings) to pea seedlings 2 days old.

than half of the C^{14} recovered was present in CO_2, homoserine and organic acids, and by 24 hours these compounds together accounted for 75 % of the C^{14}. Homoserine was the only compound in the root and shoot to become noticeably labeled, and a striking increase occurred between 12 and 24 hours, i.e. at a time when the only possible source of C^{14} was homoserine in the cotyledons. These relationships are shown in figure 4. The results show that aspartate is particularly effective as a precursor of homoserine, and that homoserine produced in the cotyledons can be transferred to the seedling proper. Glutamic acid and its amide became noticeably labeled and in fact, after 6 hours, contained more C^{14} than the substrate aspartic acid and asparagine. The labeling pattern in the glutamate so produced (C^{14} in C-1 > C^{14} in C-5) is consistent with its production from oxalacetate by the forward reactions of the tricarboxylic acid cycle.

C) Homoserine: The accumulation of homoserine under natural conditions in both cotyledons and green shoots at particular stages in their development shows that it is being produced more rapidly than it is consumed. The progressive accumulation of C^{14} from glutamate in homoserine, the temporary labeling of organic acids when this substrate was supplied, and the release of only part of the carbon as $C^{14}O_2$ are all consistent with an introduction of carbon skeletons provided by this substrate into the tricarboxylic acid cycle and a partial diversion of the oxalacetate into homoserine with little subsequent metabolism. Aspartate, as a more proximal precursor, is even more efficiently converted to homoserine, but some carbon from aspartate also enters the tricarboxylic acid cycle.

Experiments with labeled homoserine were carried out to determine how extensively it was utilized at 2 stages of development. These were at 2 days when cotyledons contain about 2.7 μmoles of homoserine and 10 days, when this value has fallen to 0.3 μmole (table III). The results, with those of parallel experiments with aspartate-U-C^{14} are shown in table VI.

Homoserine C^{14} was injected into the cotyledons of 2-day-old seedlings and in 24 hours 82 % of the C^{14} recovered was found in homoserine. In contrast to the results obtained with the other labeled compounds, very little $C^{14}O_2$ was produced, and the remainder of the C^{14} recovered was distributed in a variety of compounds. Half of the homoserine-C^{14} was recovered in the cotyledons and that in the root and shoot had apparently appeared there by translocation. This is clearly consistent with the picture

Table VI. *Comparison between the Percent Distribution of C^{14} Recovered 24 hours after supplying Homoserine-U-C^{14} and Aspartic Acid-U-C^{14} to 2- and 10-day-old Etiolated Pea Plants*

	Homoserine		Aspartic	
	2 Days*	10 Days**	2 Days***	10 Days***
Alcohol insoluble residue				
Cotyledon	5	1	8	4
Root and shoot	1	7	<1	4
CO_2	2	6	36	61
Alcohol soluble fractions				
Cotyledon				
Aspartic acid	<1	<1	<1	<1
Glutamic acid	<1	1	2	1
Asparagine + glutamine	1	1	3	2
Homoserine and its lactone	51	39	12	6
Other amino acids	2	3	<1	<1
Organic acids	2	1	8	5
Sugars	<1	<1	<1	3
Ether soluble	<1	<1	<1	<1
Root and shoot				
Aspartic and glutamic acids	<1	1	1	1
Asparagine + glutamine	<1	<1	<1	2
Homoserine and its lactone	31	37	22	7
Other amino acids	1	1	1	1
Organic acids	<1	1	<1	1
Sugars	<1	<1	<1	<1
Ether Soluble	1	<1	<1	<1
Total cpm recovered (thousands)	152	46	759	574

* Four 2-day-old seedlings were supplied with a total of 4 μmoles (1.15 μc) in 0.05 ml of solution of homoserine-U-C^{14}.

** Five 10-day-old seedlings were supplied with a total of 1.33 μmoles (0.384 μc) in 0.05 ml of solution of homoserine-U-C^{14}.

*** Five 2-day-old seedlings and five 10-day-old seedlings were each supplied with a total of 0.08 μmole (5.0 μc) in 0.05 ml of solution of aspartic acid-U-C^{14}.

arrived at from the experiments with glutamate and aspartate.

A comparable experiment with older seedlings (10 days old) whose homoserine level had already begun to fall showed virtually the same result. After 24 hours, 76% of the C^{14} recovered was still in homoserine, and although a somewhat greater proportion of this had been translocated to the root and shoot it must be remembered that this tissue is much larger in bulk at 10 days. Again no major metabolic conversion of the homoserine-C^{14} had occurred. Homoserine is thought to be an intermediate in the production of methionine and threonine, but these compounds did not become labeled.

It thus seems unlikely that the falling levels of homoserine in 10-day-old cotyledons are due to marked increases in the rate of its metabolic utilization. The parallel experiments with aspartate (table VI) suggest that a more likely explanation of the falling level of homoserine in the older cotyledons is that its rate of synthesis is lower. Thus, whereas in the younger material homoserine accounted for 28% and CO_2 for 36% of the C^{14} recovered after providing aspartate-U-C^{14}, the corresponding values in the experiment with 10-day-old material were 11% and 61%. Apparently a smaller portion of aspartate is diverted to homoserine in the older seedlings and a larger portion is dissimilated. Further experiments with older shoots would be needed to amplify this conclusion, but it appears that the striking accumulations and subsequent disappearance of homoserine represents the balance between a slow continuous turnover of homoserine, and changes in the rate of its synthesis from aspartate or other precursors.

D) Leucine: To investigate the fate of an amino acid more distantly related to probable homoserine precursors during the early stages of germination, leucine-U-C^{14} was supplied to the cotyledons of 2-day-old seedlings. After 1, 4, and 7 days, seedlings were analyzed and the distribution of C^{14} was determined (fig 5). These data show that almost all of the labeled leucine had been utilized in the first 24 hours. More than half of the C^{14} recovered at this time was in the protein of the cotyledon, and even after 7 days this figure was almost 40%. Hydroly-

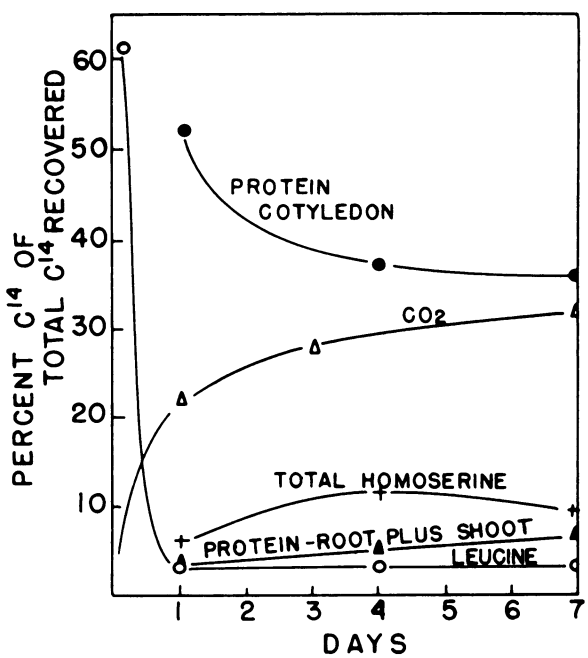

Fig. 5. Percent distribution of the C^{14} recovered 1, 4, and 7 days after supplying L-leucine-U-C^{14} (0.02 μmole, 246 mc/mmole, in 0.05 ml supplied to 5 seedlings in each experiment of 1, 4, and 7 days).

sis of the protein showed that at least 86 % of the C^{14} was in the leucine residue. It is noteworthy that leucine was incorporated in spite of the fact that a net loss of protein was occurring (fig 1). There was also some incorporation into the protein of the root and shoot. $C^{14}O_2$ accounted for 22 % of the C^{14} recovered at the end of the first day, and it was produced at a diminishing rate during the subsequent 6 days. Homoserine contained 7 % of the C^{14} recovered after 24 hours, and this value subsequently rose to over 10 %. It is therefore clear that although leucine breakdown was less extensive than that of the other amino acids, its utilization again resulted in the production of homoserine which was the biggest single repository of C^{14} in the alcohol soluble fractions of both the cotyledons and seedling proper.

Glucose: A single experiment with glucose-U-C^{14} was carried out to trace the likely fate of glucose units derived from starch breakdown in the cotyledon. The results are shown in table VII. Of the C^{14} recovered after 24 hours 75 % was found equally divided between the alcohol insoluble residue (mainly starch), CO_2 and soluble sugars. A further 10 % was recovered in homoserine while organic acids contributed 7 %. Only 5 % of the C^{14} was found as free glucose in the cotyledon, but a further 17 % was present in sucrose which contained 59 % of its C^{14} in the glucose moiety. In the root and shoot no C^{14} was found in sucrose, and the free glucose contained 4 times as much C^{14} as fructose. Again homoserine was the major soluble compound other than free sugars to become labeled, and it accounted for 10 % of the C^{14} recovered. It thus appears that at the same time as various amino acids produced from protein breakdown were being metabolized with some translocation and conversion to CO_2, glucose skeletons were also being used as respiratory substrate and as a source of amino acids, among which homoserine predominated by virtue of its low rate of turnover.

Table VII. *Distribution of C^{14} Recovered 24 Hours after Supplying Glucose-U-C^{14} to Cotyledons of 2-Day-Old Etiolated Seedlings*

A total of 2.4 μmoles of glucose-U-C^{14} (specific activity 3.9 mc/mmole) in 0.05 ml was supplied to 5 seedlings.

	cpm	%
Alcohol insoluble residue		
Cotyledon	482,000	22
Root and shoot	86,700	4
CO_2	536,000	25
Alcohol soluble fractions		
Cotyledon		
Aspartic and glutamic acid	31,800	2
Asparagine and glutamine	14,200	<1
Homoserine	129,000	6
Other amino acids	12,500	<1
Organic acids	111,000	5
Sucrose	372,000	17
Glucose	115,000	5
Ether	13,100	<1
Root and shoot		
Aspartic and glutamic acid	17,000	<1
Asparagine and glutamine	7,170	<1
Homoserine	85,000	4
Other amino acids	18,600	<1
Organic acids	33,700	2
Glucose	85,200	4
Fructose	16,800	<1
Ether	8,300	<1
Total	2,175,070	100 %

Summary

Changes in carbohydrate, protein and soluble nitrogenous components were measured in the cotyledons and root plus shoot of germinating pea seedlings. The free amino acid composition of green shoots from 3 to 25 days old and that of cotyledons at 0, 2.5 and 10 days were determined. At 7 days homoserine, which was not detected in the ungerminated seed, accounted for 70 % of the free amino acid content of the green shoots.

L-Glutamate-U-C^{14} and L-aspartate-U-C^{14} were introduced into the cotyledons of intact 2-day-old seedlings. The labeled compounds were actively metabolized, as shown by the release of $C^{14}O_2$ and conversion to organic and other amino acids, some of which were translocated to the seedling proper. Virtually no C^{14} from the added amino acids was recovered in the sugar fraction. After 24 hours homoserine was the largest single repository of C^{14} in the soluble components produced from each of the amino acids. Of the C^{14} recovered (including $C^{14}O_2$) 24 hours after introducing aspartate-C^{14} 28 % was present in homoserine while from glutamate-C^{14} the value was 22 %.

Parallel experiments with labeled aspartate and homoserine suggest that the falling level of homoserine in aging cotyledons is attributable to a declining rate of formation from its precursors.

Major portions of glucose-C^{14} and L-leucine-C^{14} supplied to cotyledons were converted to insoluble products (starch and protein respectively), but again homoserine was a major soluble product after 24 hours.

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